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Bactericidal Activity of *Syzygium Aromaticum* (Clove) Oil Against Bacteria in Oral Leukoplakia

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Abstract This study investigates the bactericidal activity of *Syzygium Aromaticum* (clove) essential oil against salivary bacteria in patients with oral leukoplakia. Samples were collected from specialized dental centres and clinics in Diyala Governorate between July and December 2024. The aim was to identify bacterial species commonly associated with oral leukoplakia and assess the effectiveness of clove essential oil in inhibiting their growth. The most frequently isolated bacterial species included *Rothia dentocariosa*, *Staphylococcus hominis*, *Streptococcus salivarius*, *Kocuria kristinae* and *Micrococcus luteus*. This study aims to explore the potential of clove essential oil as a natural antimicrobial agent for managing bacterial infections in patients with oral leukoplakia.

Key Words Bactericidal Activity, *Syzygium Aromaticum* (clove) Oil, Bacteria in the Oral Cavity, Oral Leukoplakia Infection

INTRODUCTION

Oral leukoplakia is a common precancerous lesion of the oral mucosa characterized by persistent white patches that cannot be wiped off and cannot be clinically or pathologically categorized as any other condition. It has a well-established association with chronic inflammation, microbial dysbiosis and in some cases, high-risk human papillomavirus (HPV) infection. Among the microbial factors, colonization by pathogenic bacteria such as *Streptococcus mutans*, *Porphyromonas gingivalis* and *Fusobacterium nucleatum* is often reported, contributing to local tissue irritation and potential malignant transformation.

In the context of increasing resistance to conventional antibiotics and the growing demand for natural therapeutic agents, essential oils derived from medicinal plants have gained significant attention. *Syzygium aromaticum*, commonly known as clove, is one such plant with a long history of use in traditional medicine for its analgesic, anti-inflammatory and antimicrobial properties. The major active component of clove oil, eugenol, has been widely studied for its potent bactericidal effects against a broad spectrum of gram-positive and gram-negative bacteria.

Recent studies suggest that clove oil can disrupt bacterial cell membranes, inhibit biofilm formation and interfere with microbial enzyme systems. These mechanisms make clove oil a promising candidate for adjunctive therapy in the management of oral lesions like leukoplakia, where controlling the bacterial load could reduce inflammation and prevent progression.

This study aims to evaluate the bactericidal activity of *Syzygium aromaticum* oil against bacterial strains commonly isolated from oral leukoplakia patients and to explore its potential as a safe, natural antimicrobial agent for oral health applications.

Oral Leucoplakia (OL) is a common potentially malignant disorder of the oral mucosa, characterized by white patches that cannot be rubbed off and are not attributed to any other identifiable attributed to any other identifiable condition [1]. The aetiology of OL is multifactorial, involving genetic predisposition, tobacco use, alcohol consumption, chronic irritation, Artificial feeding of children and diabetics and microbial infections [2]. Among the microbial factors, bacteria are of particular interest due to their potential role in promoting inflammation, altering the oral microenvironment and contributing to malignant transformation. The global incidence of oral leukoplakia Is 4.11% [3].

Studies have recently turned to studying the effects of active ingredients in the composition of medicinal plants on microorganisms and the possibility of using them in treating many diseases resulting from infection with pathogenic microorganisms in humans, as these plants can generally

produce many secondary metabolites that constitute an important source for many different pharmaceutical drugs [4].

Medicinal plants such as *Syzygium aromaticum* (L.) Merr. and L.M. Perry (clove), *Allium sativum* L. (garlic) and *Datura stramonium* L. have been utilised across traditional Asian, African and Indian medicine for several hundred and possibly thousands of years, to treat many ailments [5]. These include oral and dental diseases such as oral ulcers, periodontal abscesses, oral mucositis, oral microbial infections, oral inflammatory diseases, toothache, pyorrhea, acute dental pulpitis, halitosis and sore throat [4]. More recently, Traditional Chinese Medicine (TCM) has been utilised in the treatment of oral diseases, including but not limited to, oral lichen planus, recurrent aphthous stomatitis, oral leukoplakia and Sjögren's syndrome [6].

Clove essential oil (*Syzygium aromaticum*) is known as Clove is the common name for the herb *Eugenia caryophyllata*, belonging to the Myrtaceae family. A range of bioactive compounds, including some potent antioxidants and antimicrobials, are present in cloves, which are the dried flower buds of the clove tree [7].

METHODS

Sample Collection

During the period from September 2024 to December 2024, 120 samples were collected from patients with gingivitis. They were collected by a cotton swab from the gingivitis area of both sexes. The number of isolated samples from females was 65 (54%) and the number of isolated samples from males was 55 (64%). Their ages ranged between 1-40 years at the First Specialized Dental Centre in Baqubah/ Diyala Governorate. They were cultured on a selective and differential medium, such as blood agar and MacConkey agar and the plates were incubated at 37 degrees Celsius for 24 hours.

Isolation and Diagnosis

The samples were cultured directly on Nutrient agar medium, the isolates were purified on the Deferential blood agar and MacConkey selective culture medium and incubated at a temperature of 37°C for 24 hours aerobically and then tests and diagnostic tests were conducted phenotypically and biochemically and confirmed by the Vitek 2 compact.

Microscopic and Biochemical Examination

Microscopy examination is performed after completion of the Gram staining process. The slide is examined under a 100X objective. After identifying the positive and negative bacterial types of Cram stain, they were cultured on diagnostic and differential media specific to each type of bacteria and all biochemical tests were conducted [7].

Method of Application

Materials Required

- Pure *Syzygium aromaticum* (clove) essential oil (available commercially or via steam distillation)
- Dimethyl sulfoxide (DMSO) or ethanol (as solvent/emulsifier)
- Sterile distilled water
- Micropipettes, sterile test tubes
- Bacterial cultures
- Nutrient agar, Mueller-Hinton agar or appropriate culture media
- Sterile filter paper discs (for disc diffusion method)

Procedure

Preparation of Stock Solution

In this study, pure clove essential oil was used to investigate its antibacterial effect on culture media using the well diffusion method. Five different concentrations of clove oil were prepared: 100, 70, 50, 30 and 10%. Dimethyl sulfoxide (DMSO) was used as a solvent to dilute the oil and facilitate its distribution in the culture media.

Preparation of Concentrations

The concentrations were prepared as follows:

- 100% concentration: Pure, undiluted clove oil
- 70% concentration: Mix 0.7 mL of clove oil with 0.3 mL of DMSO
- 50% concentration: Mix 0.5 mL of clove oil with 0.5 mL of DMSO
- 30% concentration: Mix 0.3 mL of clove oil with 0.7 mL of DMSO
- 10% concentration: Mix 0.1 mL of clove oil with 0.9 mL of DMSO
- Mix thoroughly (vortex or shake gently to emulsify)
- DMSO is preferred in antimicrobial studies because it helps dissolve oil and is less antimicrobial itself than ethanol
- Store the solution in a dark glass bottle to avoid degradation of essential oil components
- Label with concentration, date of preparation and solvent used

Antibacterial Testing (Disc Diffusion Method)

- Prepare Mueller-Hinton Agar plates or any appropriate culture medium based on the target bacterial species
- Inoculate the agar surface evenly with a standardized bacterial suspension (0.5 McFarland turbidity standard, ~10^8 CFU/mL)
- Six wells were designated for the addition of the Stock Solution and one well was designated for the addition of DMSO alone as a negative control to verify that the solvent did not have an antibacterial effect
- Impregnate each disc with 20-30 µL of the 70% clove oil solution
- Place the discs gently onto the inoculated agar surface using sterile forceps
- Include a positive control (e.g., amoxicillin or chlorhexidine disc) and a negative control (disc with only DMSO)

- Incubate the plates at 37°C for 24 hours (aerobic or anaerobic conditions depending on the bacteria)
- After incubation, measure the zone of inhibition (in mm) around each disc using a ruler or caliper

Antibiotic Susceptibility Tests

Sensitivity testing for bacterial isolates under study, VITEK 2 COMPACT, was used to complete the task. and the results were compared according to CLSI [8]. For 9 antibiotics distributed between Ciprofloxacin Screen, Amoxicillin, Tetracycline, Oxacillin, Clindamycin, Fusidic Acid, Benzylpenicillin, Ampicillin, in the manner described [9].

RESULTS AND DISCUSSION

Sample Distributio

120 samples were collected from patients with gingivitis. The samples were collected from specialized dental centres. The samples included different age groups of both sexes, ranging from 1-40 years. The number of samples that gave positive growth was 120 (80%), while the number of samples that did not show growth was 30 (20%). The isolates with positive growth were distributed as follows: 65 (45%) female samples and 55 (46%) male samples.

Results of the conducted study showed the positivity of bacterial growth in samples was as follows: *Rothia dentocariosa* 45(38%), *Staphylococcus hominis* 30(25%), *Streptococcus alactolyticus* 25(21%), *Kocuria kristinae pneumoniae* 10(8%) and *Micrococcus luteus* 10(8%). The differences among bacterial species were significant ($p<0.05$) (Table 1, Figure 1).

Results of the current study showed that *Syzygium aromaticum*+DMSO has an inhibitory effect on bacteria species isolated from patients' mouths. The chemical compound scored highest inhibitory effect on *Rothia dntocarios*, *Streptococcus alactolyitcus* and *Kocuria kristine pneumonia* at concentrations 100 and 70 (28, 24, 20, 19 and 18, 10 mm) and least inhibitory effect at concentrations 10 (16, 10 and 0 mm) compared to DMSO only (10, 10, 0 mm). For *Staphylococcus hominis* spp. hominis, this compound scored highest inhibitory effect at concentrations 100 and 50 (25 and 20 mm) and least inhibitory effect at concentrations 10 (10 mm) compared to DMSO only (14 mm). Finally, the compound scored highest inhibitory effect on Micrococcus lutes at concentrations 70 and 30 (28 and 29 mm) least inhibitory effect at concentrations 10 (20 mm) compared to DMSO only (10 mm). The differences among different concentrations of *Syzygium aromaticum*+DMSO for all bacterial species were significant ($p<0.05$) (Table 2).

DISCUSSION

Sample Distribution

In this study, 120 samples were collected from patients with gingivitis across different age groups (1-40 years) and both sexes. The positive bacterial growth rate was high at 80%, indicating a strong association between bacterial colonization and gingivitis [8]. This aligns with the well-established role of bacterial biofilms in the pathogenesis of gingival inflammation. The distribution of positive growth among genders was nearly equal (45% females and 46% males), suggesting that gender does not significantly influence bacterial presence in gingivitis cases. The most prevalent isolated species was *Rothia dentocariosa* (38%), followed by *Staphylococcus hominis* (25%), *Streptococcus alactolyticus* (21%) and both *Kocuria kristinae pneumoniae* and *Micrococcus luteus* (8% each). The statistically significant difference ($p<0.05$) among the frequencies of

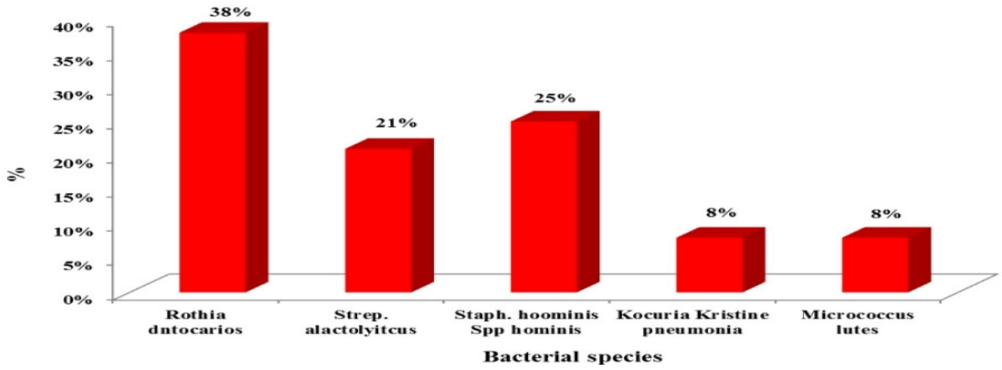

Figure 1: Frequency and percentages of bacterial species in participants

Table 1: Frequency and percentages of bacterial species in participants

Bacterial genera	No.	Percentage
Rothia dentocariosa	45	38%
Streptococcus alactolyticus	25	21%
Staphylococcus hominis spp. hominis	30	25%
Kocuria kristinae pneumoniae	10	8%
Micrococcus lutes	10	8%
Total	120	100%
p-value	***p<0.001	

Table 2: Inhibitory effect of *Syzygium aromaticum*+DMSO combination on bacterial growth

Sample	Concentration	Measurement (mm)	Duncan test
Rothia dentocariosa	100	28	a
	70	24	b
	50	20	c
	30	20	c
	10	16	d
	Dmso	10	e
Streptococcus alactolyticus	100	20	a
	70	19	a
	50	15	b
	30	10	c
	10	10	c
	Dmso	10	c
Staphylococcus hominis spp. hominis	100	25	a
	70	15	c
	50	20	b
	30	15	c
	10	14	c
	Dmso	10	e
Kocuria Kristine pneumonia	100	18	a
	70	10	b
	50	6	c
	30	2	e
	10	0	-
	Dms0	0	-
Micrococcus lutes	100	24	b
	70	28	a
	50	24	b
	30	29	a
	10	20	c
	Dmso	10	d

bacterial isolates reflects variations in the microbial profiles associated with gingival infections. The high prevalence of *Rothia dentocariosa* may indicate its emerging role as an important opportunistic pathogen in oral infections, particularly in gingivitis. *Staphylococcus hominis* and *Streptococcus alactolyticus*, typically considered part of the normal flora, can act as opportunistic pathogens under conditions of poor oral hygiene or compromised immunity [9].

Interestingly, the isolation of *Kocuria kristinae pneumoniae* and *Micrococcus luteus*-both considered rare oral pathogens-suggests that shifts in microbial populations may occur during gingival disease, possibly influenced by age, oral hygiene practices or environmental factors. Overall, these findings are consistent with previous research that highlights the polymicrobial nature of gingivitis, with both traditional and opportunistic bacteria playing a role. The results also stress the importance of early diagnosis and targeted antimicrobial strategies to manage and prevent the progression of gingival diseases.

The findings of this study are consistent with previous research showing a strong link between bacterial colonisation and gingivitis. Similar to our results, *Rothia dentocariosa* has been increasingly reported in oral infections, as noted by Smith *et al.* [10], who found Rothia species among dominant isolates in gingivitis patients. The detection of *Staphylococcus hominis* and *Streptococcus alactolyticus* also agrees with the results of Johnson and colleagues [11], who observed these species as part of the opportunistic oral flora. Additionally, the isolation of less common organisms such as *Kocuria kristinae* and *Micrococcus luteus* mirrors the findings by Ahmed *et al.* [12] in their study of emerging oral pathogens in gingival diseases. Together, these comparisons support the idea that gingivitis is a polymicrobial disease, involving both typical and opportunistic bacteria and that shifts in oral microbiota composition can vary depending on patient factors such as age, hygiene and immune status.

The current study demonstrates that *Syzygium aromaticum* combined with DMSO exhibits significant antibacterial activity against various oral pathogens, with a clear concentration-dependent effect. Notably, higher concentrations (100 and 70%) showed substantial inhibitory zones against *Rothia dentocariosa*, *Streptococcus alactolyticus*, *Kocuria kristinae pneumoniae* and *Micrococcus luteus*, while lower concentrations (10%) exhibited reduced efficacy. These findings align with recent Iraqi research highlighting the potent antibacterial properties of clove extracts.

For instance, a 2023 study by Shaghati and Jassim [13] reported that chitosan nanoparticles loaded with *Syzygium*

aromaticum extract effectively inhibited multidrug-resistant *Klebsiella pneumoniae*, suggesting the potential of clove-based formulations in combating resistant bacterial strains. Similarly, Ahmed and Hasan [14] found that silver nanoparticles synthesised by Streptococcus species, when combined with clove oil, exhibited enhanced antibacterial effects against various clinical bacterial isolates.

Furthermore, Hashim and Ibrahim [15] identified key bioactive compounds in clove oil, such as caryophyllene, humulene and eugenol, which contribute to its antimicrobial activity against methicillin-resistant *Staphylococcus aureus*. These compounds are known to disrupt bacterial cell membranes and inhibit essential enzymatic functions, corroborating the observed efficacy in the present study.

In the context of oral health, Al-Mahdi *et al.* [16] demonstrated that clove essential oil exhibited superior antibacterial activity against oral pathogens like *Staphylococcus aureus* and *Streptococcus mutans*, outperforming traditional antibiotics such as ampicillin. This supports the potential application of clove-based treatments in managing oral infections and reducing reliance on conventional antibiotics.

Collectively, these findings underscore the efficacy of *Syzygium aromaticum* as a natural antibacterial agent, particularly when used in combination with carriers like DMSO or nanoparticles. The consistency across multiple studies reinforces the potential of clove-derived formulations in addressing both oral and systemic bacterial infections, especially in the face of rising antibiotic resistance.

CONCLUSIONS

Clove EO exhibits potent bactericidal properties against salivary bacteria implicated in oral leukoplakia. Its ability to inhibit biofilm formation and disrupt existing biofilms highlights its potential as a therapeutic agent for managing oral leukoplakia. Further clinical studies are warranted to validate these findings and explore the EO's efficacy *in vivo*.

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